International Journal of Medicine and Pharmaceutical Sciences (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 4, Issue 2, Apr 2014, 139-150

THE VIRTUAL MATRIX BASED METHODS WITH METHOD BASED ON THE SUPPORT VECTOR MACHINES AND ARTIFICIAL NEURONAL NETWORK

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ABSTRACT

Propred –I and Propred are the matrix based method while CTL Pred is a method based on the combined approach of support vector machine and artificial neuronal network. Promiscuous T-cell epitopes make ideal targets for vaccine development. PfM18AAP is a malarial antigenic protein responsible for rupturing of RBC cells. The PfM18AAP of Plasmodium falciparum malaria is a metallo-aminopeptidase that shows highly restricted specificity for peptides with an N-terminal Glu or Asp residue. Thus, it is responsible in effecting the complete degradation or turnover of proteins, such as host hemoglobin, which provides a free amino acid pool for the growing parasite Thus, used method allows us identification of MHC class I binders (peptides binding with many MHC alleles) & MHC-II binders having proteasomal cleavage site at C-terminus. The user-friendly result display format (HTML-II) can assist in locating the promiscuous MHC binding regions from antigen sequence.

KEYWORDS: T-Cell, Vaccines, RBC Cells, MHC Class I &II

INTRODUCTION

Malaria, caused by Plasmodium falciparum, is a major public health concern and has now been identified as one of the emerging infectious diseases worldwide mainly in sub Saharan areas. The symptoms mainly include headache, fever, chill, sweating, dry cough, spleen enlargement, muscular fatigue &pain, back pain, nausea, vomiting, etc. One of the crucial steps in designing subunit vaccine for diseases like malaria involves identification of antigenic peptides that can stimulate cytotoxic T lymphocytes (CTLs). The binding of antigenic peptide to MHC class I molecule is a prerequisite for their recognition by CTLs (Cresswell*et al* 1999). (1). T-cell epitope identification is a challenging immune informatic problem within vaccine design. To be an epitope, a peptide should bind a major histocompatibility complex (MHC) protein. (2). There are two discrete classes of MHC molecules: (i) MHC class I presents endogenous peptides; and (ii) MHC class II presents exogenous peptides. The process of MHC class I antigen presentation involves protein degradation, peptide transport to the endoplasmic reticulum, peptide–MHC binding and export of peptide–MHC complexes to the cell surface for recognition by CD8 T cells. T cells are activated when the T-cell receptor recognizes a specific peptide–MHC complex, and in this way identify cells infected by intracellular parasites and mount appropriate immune responses against them. The peptides involved in specific peptide–MHC complexes triggering T-cell recognition (T-cell epitopes) are important tools for the diagnosis and treatment of infectious. Because T-cell epitopes are subsets of MHC-binding

peptides, precise identification of portions of proteins that can bind MHC molecules is important (4). for the design of vaccines and immunotherapeutic (3). recently, development of several immune informatics and computational biology tools are useful for identification of Antigenic regions (epitopes) in the protein sequences which can accelerate the wet laboratory practices. These tools have been developed on the basis of existing and validated data with specific algorithms. [5] An epitopes are also known as antigenic determinant in the protein sequences which is recognized by the major histocompatibility complex (MHC) molecules (4).

MATERIALS AND METHODS

Glycoprotein Sequence

Plasmodium falciparum glycoprotein is a major immunogenic protein of Malaria. It is 570 amino acids long and is frequently abbreviated to pfM18AAP protein. Plasmodium falciparum protein pfM18AAP sequence was retrieved from the NCBI Entrez protein database.

Prediction of MHC Class-I Binding Peptides: The Use of Propred I

The prediction of promiscuous MHC Class-I binding peptides was done by using ProPred I. ProPred I is an on-line tool for the prediction of peptide binding to MHC Class-I alleles. This is a matrix-based method that allows the prediction of MHC binding sites in an antigenic sequence for 47 MHC class-I alleles. The server represents MHC binding regions within an antigenic sequence in user-friendly formats. These formats assist user in the identification of promiscuous MHC binders in an antigen sequence that can bind to large number of alleles. ProPred1 also allows the prediction of the standard proteasome and immunoproteasome cleavage sites in an antigenic sequence. This server allows identification of MHC binders, who have the Cleavage site at the C terminus. The simultaneous prediction of MHC binders and Proteasome cleavage sites in an antigenic sequence leads to the identification of potential T-cell epitopes. Server is available at http://www.imtech.res.in/raghava/propred1/. Mirrorsite of this server is available at http://bioinformatics.uams.edu/mirror/propred1/.

Matricesand document on server are available athttp://www.imtech.res.in/raghava/propred1/page2.html.

The pfM18AAP protein sequence (570 amino acids) was analyzed at a threshold setting of 10%. A total of 48 alleles of Human Leukocytic Antigens were taken into consideration. Proteosome and immunoproteosome filters were set on a threshold of 5%. Results were taken in tabular and HTML formats. The subsequence analysis was also done by keeping the proteome filters ‘on’.

Prediction of MHC Class-II Binding Peptides: The Use of Propred

The prediction of promiscuous MHC class-I binding peptides was done by using ProPred (Singh & Raghava, 2003). ProPred is a graphical web tool for predicting MHC class II regions in antigenic protein sequences. The server implements matrix based prediction algorithm employing amino acid / position coefficient table deduced from literature. The predicted binders can be visualized either as peaks in graphical interface or as colored residues in HTML interface. The server is a useful tool to locate promiscuous binding region that can bind to several HLA-DR alleles. The pfM18AAP protein sequence (570 amino acids) was analyzed at a threshold setting of 10%. A total of 51 alleles of HLA-DR were taken into consideration. The server is available at http://www. imtech.res.in/raghava/propred/. Supplementary information is available at http://www.imtech.res.in/raghava/propred1/page2.html.

Analysis of the Antigenic Peptides by CTLPred

The method has the ability to predict the CTL epitopes from the antigenic sequence on the basis of Artificial Neural Networks and Support Vector Machine (Bhasin & Raghava, 2003). The server also allows consensus and combined prediction by using above two prediction methods. The user can only apply one prediction approaches at a single run. The consensus and combined prediction results in enhancement of specificity and sensitivity respectively as compared to individual approaches like ANN and SVM. The user can vary the cutoff score for all prediction approaches. In this study a combined approach was used to determine the antigenic peptides. The results were compared with the results of the ProPred 1 &ProPred to see whether the antigenic peptides predicted by both the approaches are same or not. The method can be accessed freely from URL http://www.imtech.res.in/raghava/ctlpred. The method can be accessed through web Browser like Netscape, internet explorer, etc.

Analysis of the Conserved Domains in pfM18AAP Protein

Domains can be thought of as distinct functional and/or structural units of a protein. These two classifications coincide rather often, as a matter of fact, and what is found as an independently folding unit of a polypeptide chain also carries specific function. Domains are often identified as recurring (sequence or structure) units, which may exist in various contexts. Molecular evolution may have utilized such domains as building blocks, recombined in different arrangements to modulate protein function. We define conserved domains as recurring units in molecular evolution, whose extents can be determined by sequence and structure analysis. Conserved domains contain conserved sequence patterns or motifs, which allow for their detection in polypeptide sequences. The distinction between domains and motifs is not sharp, however, especially in the case of short repetitive units. Functional motifs are also present outside the scope of structurally conserved domains. The CD database is not meant to systematically collect such motifs. Conserved Domain analysis can be done from URL http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml (Marchler*et al.*, 2002)

Prediction of Transmembrane Helices in pfM18AAP Protein

Analysis of the G protein amino acid was done by TMHMM server v.2.0. (Krough*et al.,* 2001) The server is hosted at the Center for Biological Sequence Analysis, Technical University of Denmark. The method can be accessed freely from URLhttp://www.cbs.dtu.d/services/TMHMM/.

RESULTS & DISCUSSIONS

Glycoprotein Sequence

The FASTA format number of the pfM18AAP protein is GI/124507185|. The sequence is 570

amino acids long. The sequence is shown below.

>GI/124507185|ref|XP_001352189.1| M18 aspartiyl amino peptidase [Plasmodium falciparum 3D7]

MDKKAREYAQDALKFIQRSGSNFLACKNLKERLENNGFINLSEGETWNLNKNEGYVLCKENRNICGFFV
GKNFNIDTGSILISIGHIDSCALKISPNNNVIKKKIHQINVECYGSGLWHTWFDRSLGLSGQVLYKKGNKV
EKLIQINKSVLFLPSLAIHLQNRTRYDFSVKINYENHIKPIISTTLFNQLNKCKRNNVHHDTILTTDTKFSHK
ENSQNKRDDQMCHSFNDKDVSNHNLDKNTIEHLTNQQNEEKNKHTKDNPNSKDIVEHINTDNSYPLLYL
LSKELNCKEEDILDFELCLMDTQEPCFTGVYEEFIEGARFDNLLGSFCVFEGFIELVNSIKNHTSNE

NTNHTNNITNDINDNIHNNLYISIGYDHEEIGSLSEVGARSYCTKNFIDRIISSVFKKEIHEKNLSVQEIYGN
LVNRSFILNVDMAHCSHPNYPETVQDNHQLFFHEGIAIKYNTNKNYVTSPLHASLIKRTFELYYNKYKQQ
IKYQNFMVKNDTPCGSTVGSMVAANLSMPGIDIGIPQLAMHSIREIAAVHDVFFLIKGVFAFYTYYNQVL
STCVHDK

Prediction of MHC Class-I Binding Peptides: The Use of ProPred 1

The prediction of promiscuous MHC class-I binding peptides was done by using ProPred1 (Singh & Raghava, 2003). The results were taken in HTML and tabular forms. The pfM18AAP protein sequence (570 amino acids) was analyzed at a threshold setting of 10%. A total of 48 alleles of Human Leukocytic Antigens were taken into consideration. Proteosome and immune proteosome filters were set on a threshold of 5%. Results were taken in tabular and HTML formats. The subsequence analyses were also done by keeping the proteome filters 'on'. The MHC class I binding peptides was scored in range of 16-4000. Only peptides with a score of 240 or more than 240 were selected for analysis. These selected for the analysis of the consensus MHC I binding epitopes from the HTML format results. The results are given in table 2. Analyses of the HLA Class I binding peptides of the Glycoprotein (gi|124507185) of antigenic protein on ProPred 1 lead to the recognition of a total of 516 nonamers and their peptide score ranged 16-4000. Upon their analyses a total of 7 peptides with peptide scores of more than 240 were selected to determine consensus MHC class I binding peptides. Out of these 7 peptides, 5 were predicted to be non bindersto few of the 48 alleles of the HLA molecule taken into consideration. And, 1 peptides were partial binder to few HLA molecules. Only 1 peptides were able to bind to all of the 48 alleles of the HLA molecules. These peptides are non amer KSVLFLPSL(149-157.The peptides, KSVLFLPSL (149-157)has a peptide score of 720 with allele HLA-Dd. This peptide have consensus for binding with all 48 alleles In peptide, KSVLFLPSL amino terminal has lysine at position P1which is positively charged amino acid and two serine are present and P2, P8 respectively at carboxy terminal. At P3 valineis present which is hydrophobic amino acid. At P4, P6 and P9 leucine is present which is sparingly soluble in water. At P5 phenylalnine is present which is aromatic amino acid. Both valine and leucine are hydrophobic amino acids which fulfils the criteria of MHC class I binding peptides. Together with hydrophobic amino acids, charged amino acid also contributes towards the interaction with the MHC pockets. Out of nine amino acid, four amino acid which give sufficient reason to undertake experimental studies with this peptide. (Table 1)

Prediction of MHC Class-II Binding Peptides: The Use of ProPred

The prediction of promiscuous MHC class-I binding peptides was done by using ProPred (Singh & Raghava, 2003). The results were taken in HTML and graphical forms. The pfM18AAP protein sequence (570 amino acids) was analyzed at a threshold setting of 10%. A total of 51 alleles of HLA - DR were taken into consideration. The consensus MHC II binding epitopes were selected from the HTML format results. The results are given in table 3. Analyses of the HLA class II binding peptides of the glycoprotein (gi|**124507185**) of Malarial protein on ProPred lead to the recognition of a total of five peptides of different length(1 was 18mer, 3 were 9 mer, and 2 were 10mer). Out of these, three peptides LIQINKSVLFLPSLAIHL (18 mer, 144-161), LLYLLSKELN (10 mer, 280-290) and VGSMVAANL (9 mer, 420-438) binds with all of the 51alleles of HLA-DR molecules. In peptide LIQINKSVLFLPSLAIHL, amino terminal has a leucine at P1 position which is hydrophobic amino acid, at P2, P4& P16 is a isoleucine is present, at P3 glutamin, P5 asparagine, both asparagine and glutamin are polar amino acid, P6 lysine which is a positively charged amino acid, P7 & P13 serine is

present, P8, P9, P11P14&P18 is leucine, P10 phenylalanine both leucine and phenylalanine is hydrophobic amino acids, at P12prolinea hydrophobic amino acid is present, P15 is alanine is present, P17 is histidine, a positively charged amino acid is present. The core of peptide sequence is hydrophobic in nature. In peptide sequence LLYLLSKELN, at P1, P2, P4, P5& P9 leucine a hydrophobic amino acid is present, at P3 tyrosin a polar amino acid is present, at P6 serine is present, at P7 positively charged lysine is present, at P8 negatively charged glutamic acid is present, at P10 polar aspargine is present. Similarly LVNSIKNHT(9mer, 339-347)is a binder of all of the HLA-DR allelesare therefore all three peptide sequence are consensus MHC II binding epitopes of the pfM18AAP protein of Plasmodium falciparum. (Table 2)

Analysis of the Antigenic Peptides by CTLPred

The method has the ability to predict the CTL epitopes from the antigenic sequence on the basis of Artificial Neural Networks and Support Vector Machine (Bhasin & Raghava, 2003). Peptide sequences **'**KSVLFLPSL (149-157) which were predicted to be a MHC I binding consensus sequence by Properd-I are also found to be antigenic as predicted by CTLPred (Figure 1).

Peptide sequences LIQINKSVLFLPSLAIHL (18 mer, 144-161), LLYLLSKELN(10 MER, 280-290) and VGSMVAANL (9 mer,420-438) which were predicted to be a consensus MHC II binding epitopes by ProPerd are also found to contain antigenic peptides as predicted by CTLPred (Figure 2a, 2b & 2c) Peptide sequence 'LLYLLSKELN' (280-290) contains two antigenic nonamers i.e LLYLLSKEL(280-289) & LYLLSKELN (281-290). Peptide sequence' LIQINKSVLFLPSLAIHL' (144-161) contains three antigenic nonamersi.e QINKSVLFL (146-154), IQINKSVLF (147-153) & INKSVLFLP (147-155). Peptide sequence 'KSVLFLPSL' (149-157) which was predicted to be a MHC I binding consensus sequence by Propred-I is also found to be antigenic as predicted by CTL Pred. (Figure 1)

Peptide sequence 'LIQINKSVLFLPSLAIHL (144-161) which was predicted to be a consensus MHC II binding sequence by Propred is also found to contain antigenic peptides as predicted by CTLPred (Figure 2a).

Peptide sequence 'LLYLLSKELN (280-290) which was predicted to be a consensus MHC II binding sequence by ProPerd is also found to contain antigenic peptides as predicted by CTLPred (Figure 2b). Peptide sequence 'VGSMVAANL' which was predicted to be a consensus MHC II binding sequence by ProPerd is also found to contain antigenic peptides as predicted by CTLPred (Figure 2c).

Analysis of the Conserved Domains in the pfM18AAP Protein

The Conserved Domain analysis was done from URLhttp://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml. As we can observe from the Figure 3(a) that in MHC class II binding consensus peptide 'LLYLLSKELN'(280-290) has conserved amino acids L(P1), L(P2), L(P4), K(P7), E(P8), L(P9). Also another MHC class II binding consensus peptide 'VGSMVAANL' (420-438) has conserved amino acids at G(P2), N(P8), & L(9). As we can observed from Figure 3(b). that in MHC Class I binding consensus peptide 'KSVLFLPSL'(149-157) has conserved amino acids K(P1), L(P4), P(P7) & L(P9). Also another MHC Class II binding consensus peptide 'LIQINKSVLFLPSLAIHL'(144-161) has conserved amino acids L(P1), I(P2), I(P4), N(P5), K(P6), L(P9), P(P12), L(P14), A(P15), I(P16), H(P17), L(P18).

Prediction of Transmembrane Helices in pfM18AAP Protein

Analysis of the pfM18AAP protein amino acid was done by TMHMM server v.2.0 (Krough*et al.*, 2001) at

http://www.cbs.dtu.d/services/TMHMM/. This analysis was done to identify the location of the different amino acid residues i.e. whether they lay above the membrane towards the N terminus or they are transmembranal or towards the C terminus. The results are shown in Figure 4 & table 3a, 3b, 3c & 3d. So, predicted consensus MHC class I&II binding peptide KSVLFLPSL' (149-157), LVNSIKNHT' (339-347), LIQINKSVLFLPSLAIHL' (144-161), LLYLLSKELN' (280-289) lays on the surface towards the N terminus outside.

CONCLUSIONS

- The search for the consensus MHC class I & II binding sequences for use in aVaccine and a diagnostic vaccine resulted in the finding of four peptides. These peptides are:-

MHC Class I Binding Peptides

KSVLFLPSL' (149-157),

MHC Class II Binding Peptides

LVNSIKNHT (339-347)

LIQINKSVLFLPSLAIHL (144-161)

LLYLLSKELN (280-289)

- ProPred 1 and ProPred which are based on the virtual matrices and CTLPred which is based on the support vector machines and artificial neuronal networks, predicted for the same peptides in the present study. CTLPred predicted antigenic nonamers are present in the T-H cell epitopes predicted by ProPred. Also the T-c cell epitopes predicted by CTLPred are same as predicted by ProPred 1. These findings can solve the problem of prediction of MHC binding peptides by the use of ProPred 1, ProPred for epitope prediction and then confirming these findings with the results of CTLPred.
- The pfM18AAP protein of the Plasmodium falciparum is a N-teminal outside with distribution of MHC class I & II binding peptides in all regions of the pfM18AAP protein the study has predicted and narrowed down the search for peptides to the extent that only 1 out of 516 MHC class I binding nonamer peptides and 3 out of 416 MHC class II binding nonamer peptides were predicted. These databases are helpful in designing of peptide prediction methods. The findings of the present study can be extended by synthesis of peptides and testing of these peptides *in vivo* and *ex vivo*

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10. VLADIMIR BRUSIC, 1 NIKOLAI PETROVSKY, 2 GUANGLAN ZHANG1 a n d VLADIMIR B BAJIC1 Prediction of promiscuous peptides that bind HLA class I molecules

APPENDICES

Supplementary Material

Table 1: Analyses of the HLA Class I Binding Peptides of the Glycoprotein (gi/124507185) of Plasmodium Falciparum

S.NO.	MHC Allele	Sequence	Peptide Position	Peptide Score	Binder/Non Binder	Non Binder to Hla	Partial Binder to Hla	Binder to All Hla	Consensus
1	HLA-A20 Cattle	LLYLLSKEL	280	4000	PREDICTD BINDER	HLA-B*0702			
2	HLA-B*2705	IQRSGSNFL	16	3000	PREDICTD BINDER		HLA-A1		
3	MHC-Kd	AAVHDVFFL	540	2764.8	PREDICTD BINDER	HLA-B*0702			
4	HLA-Dd	KSVLFLPSL	149	720	PREDICTD BINDER			BIND TO ALL	
5	HLA-CW* 401	GIDIGIPQL	523	330	PREDICTD BINDER	HLA-B*0702,			
						HLA-B*51,			
						HLA-B*5401,			
						HLA-B*5301			
6	HLA-A24	KGNKLVEKL	540	240	PREDICTD BINDER	HLA-B*51,		320	PREDICTED BINDER
						HLA-B*5301			
7	HLA-A68.1	DILDFELCL	294	240	PREDICTD BINDER	HLA-B*0702			

Table 2: Analyses of the HLA Class II Binding Peptides of the Glycoprotein (gi/124507185) of Plasmodium Falciparum

Sequences	Peptide Length	Peptide Position	Binder/ Non Binder	Binding to HLA		
				Non Binder	Partial Binder	Binder
LKFIQRSGS	9 mer	13-21			DRBI-0703, DRBI-0701	Bind to all except partial binder
LIQINKSVLFLPSLAIHL	18 mer	144-161	Binder			Bind to all
LLYLLSKELN	10 mer	280-290	Binder			Bind to all
LVNSIKNHT	9 mer	339-347	Binder			Bind to all
VGSMVAANL	9 mer	420-438			DRBI-0301	Bind to all except partial binder

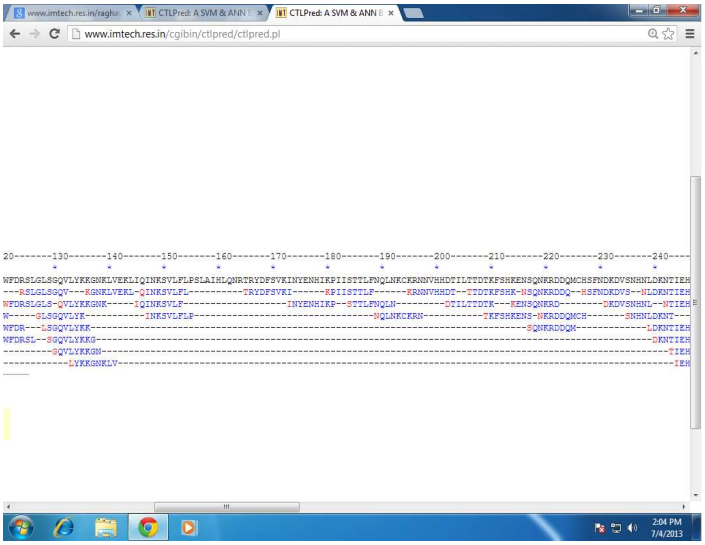

Figure 1: Peptide Sequence 'KSVLFLPSL' (149-157) Which was Predicted to be a MHC I Binding Consensus Sequence by Propred-I is Also Found to be Antigenic as Predicted by CTL Pred

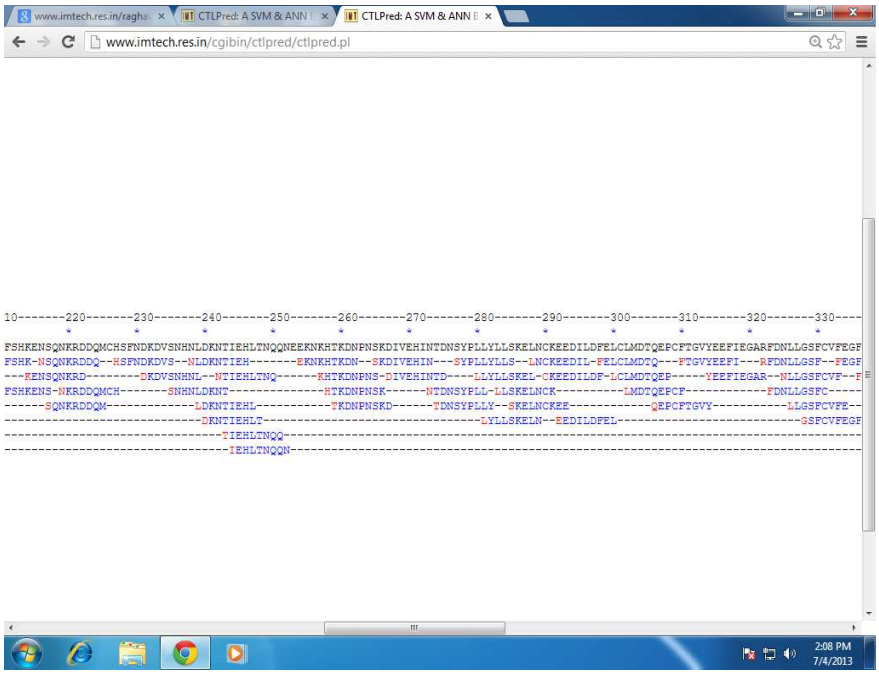

Figure 2a: Peptide Sequence 'LIQINKSVLFLPSLAIHL (144-161) Which was Predicted to be a Consensus MHC II Binding Sequence by Propred is Also Found to Contain Antigenic Peptides as Predicted by Ctlpred

Figure 2 b Peptide sequence 'LLYLLSKELN (280-290) which was predicted to be a consensus MHC II binding sequence by ProPerd is also found to contain antigenic peptides as predicted by CTLPred

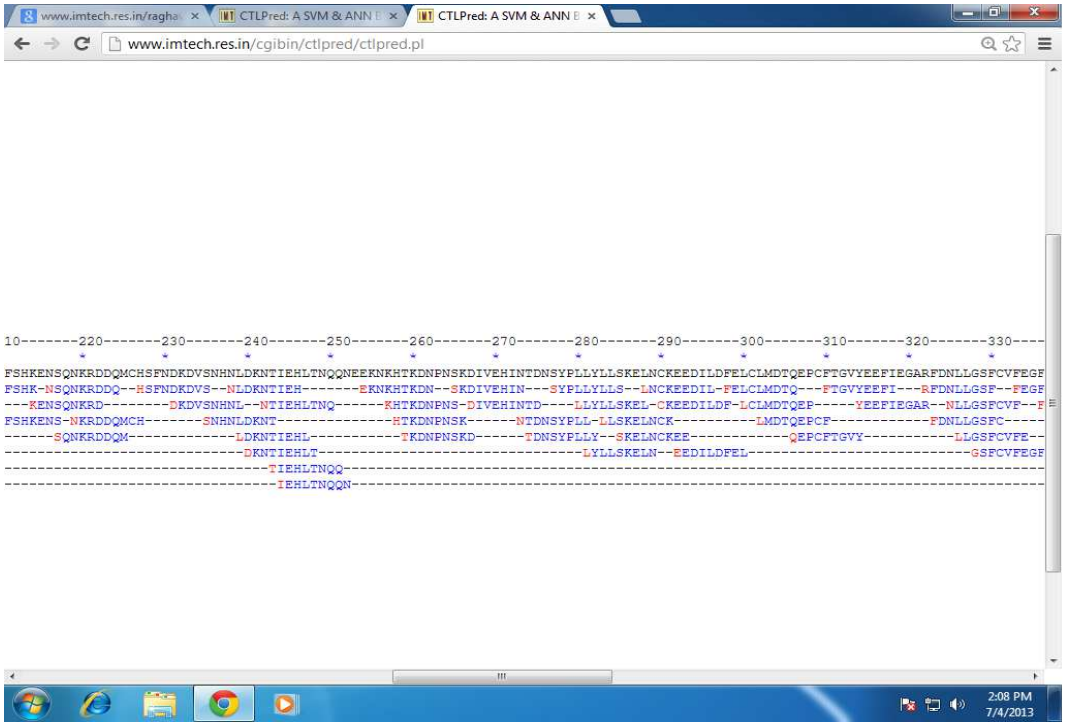

Figure 2b

Figure 2c: Peptide Sequence 'VGSMVAANL' Which was Predicted to be a Consensus MHC II Binding Sequence by Properd is Also Found to Contain Antigenic Peptides as Predicted by Ctlpred

Figure 3a: CDD Analysis of the MHC Class II Binding Peptides

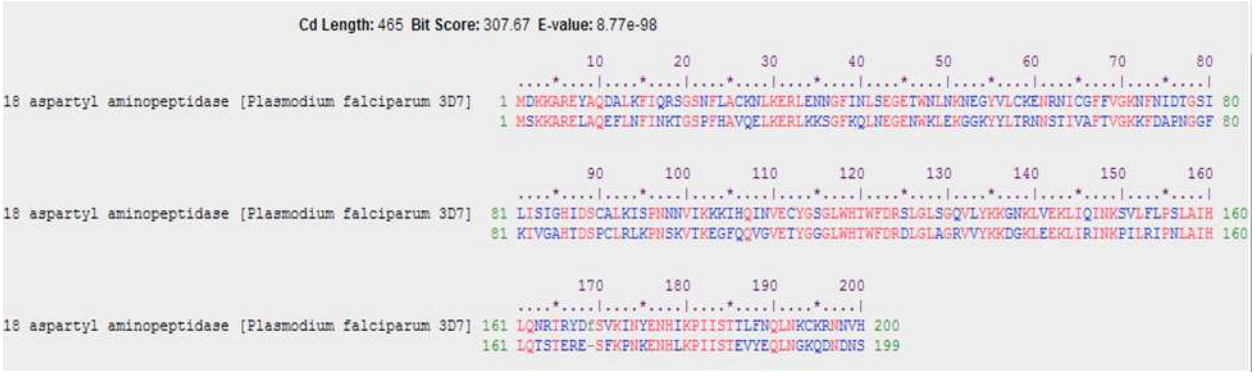

Figure 3b: CDD Analysis of the MHC Class I & II Binding Peptides

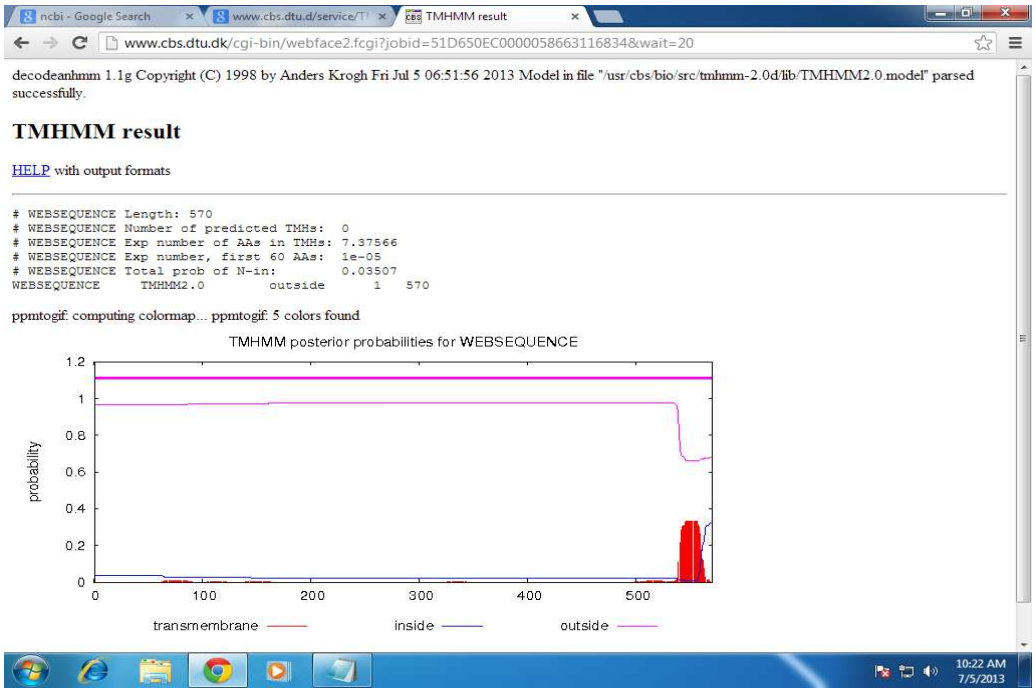

Figure 4: Graphical Output of the Result of Prediction of Transmembrane Helices in Malarial Glycoprotein from TMHMM Server v. 2.2

Table 3(a): Transmembranal Profile of Predicted Consensus MHC Class I Binding Peptide 'KSVLFLPSL' (149-157)

S. NO.	AA	Inside	Membrane	Outside
149.	K	0.02399	0.00265	0.97336
150.	S	0.02399	0.00265	0.97336
151.	V	0.02399	0.00266	0.97336
152.	L	0.02399	0.00266	0.97336
153.	F	0.02399	0.00266	0.97336
154.	L	0.02399	0.00266	0.97336
155.	P	0.02399	0.00266	0.97336
156.	S	0.02399	0.00266	0.97336
157.	L	0.02399	0.00266	0.97336

Table 3(b): Transmembranal Profile of Predicted Consensus MHC Class II Binding Peptide 'LVNSIKNHT' (339-347)

S. NO.	AA	Inside	Membrane	Outside
339.	L	0.02400	0.00017	0.97584
340.	V	0.02400	0.00016	0.97584
341.	N	0.02401	0.00015	0.97584
342.	S	0.02401	0.00015	0.97584
343.	I	0.02403	0.00013	0.97584
345.	K	0.02414	0.00002	0.97585
346.	N	0.02414	0.00001	0.97585
347.	H	0.02415	0.00000	0.97585
348.	T	0.02415	0.00000	0.97585

Table 3(c): Transmembranal Profile of Predicted Consensus MHC Class II Binding Peptide 'LIQINKSVLFLPSLAIHL' (144-161)

S. No.	AA	Inside	Membrane	Outside
144.	L	0.02436	0.00228	0.97336
145.	I	0.02405	0.00260	0.97336
146.	Q	0.02403	0.00262	0.97336
147.	I	0.02400	0.00264	0.97336
148.	N	0.02400	0.00265	0.97336
149.	K	0.02399	0.00265	0.97336
150.	S	0.02399	0.00265	0.97336
151.	V	0.02399	0.00266	0.97336
152.	L	0.02399	0.00266	0.97336
153.	F	0.02399	0.00266	0.97336
154.	L	0.02399	0.00266	0.97336
155.	P	0.02399	0.00266	0.97336
156.	S	0.02399	0.00266	0.97336
157.	L	0.02399	0.00266	0.97336
158.	A	0.02399	0.00265	0.97336
159.	I	0.02399	0.00264	0.97338
160.	H	0.02399	0.00245	0.97356
161.	L	0.02399	0.00238	0.97363

Table 3(d): Transmembranal Profile of Predicted Consensus MHC Class II Binding Peptide 'LLYLLSKELN' (280-289)

S. NO.	AA	Inside	Membrane	Outside
280.	L	0.02400	0.00000	0.976
281.	L	0.02400	0.00000	0.976
282.	Y	0.02400	0.00000	0.976
283.	L	0.02400	0.00000	0.976
284.	L	0.02400	0.00000	0.976
285.	S	0.02400	0.00000	0.976
286.	K	0.02400	0.00000	0.976
287.	E	0.02400	0.00000	0.976
288.	L	0.02400	0.00000	0.976
289.	N	0.02400	0.00000	0.976

Out of 570 amino acids, following pattern of location of amino acids in pfM18AAP protein of Plasmodium falciparum was seen:-

Outside, from transmembrane to N terminal: 1-348